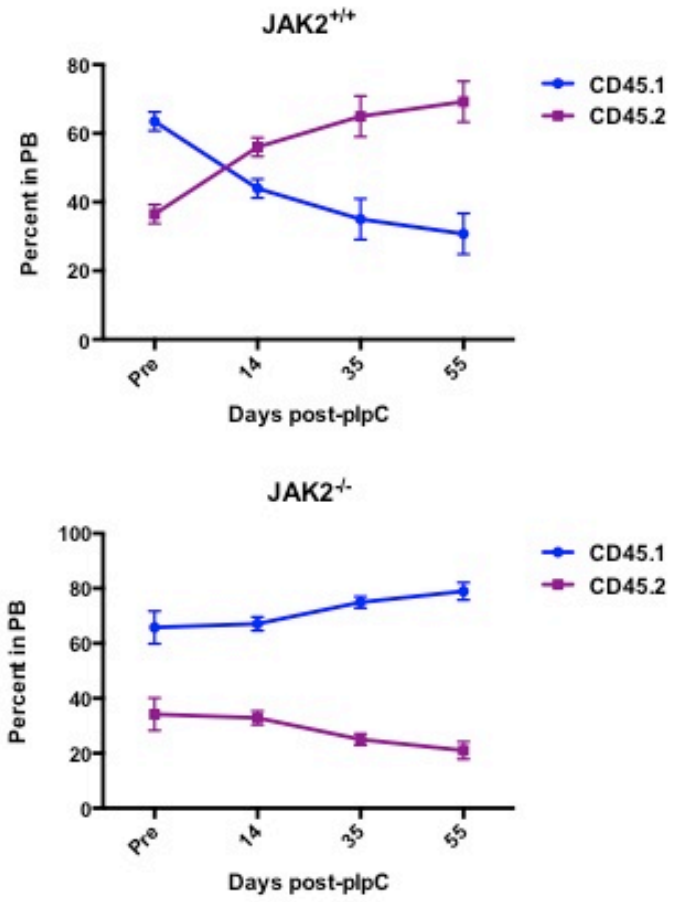
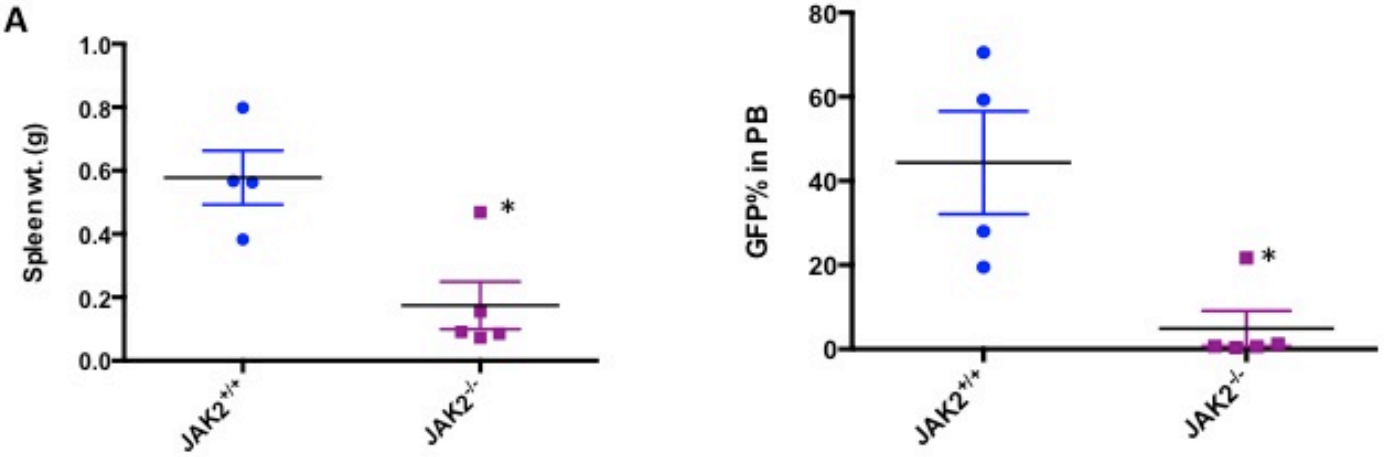
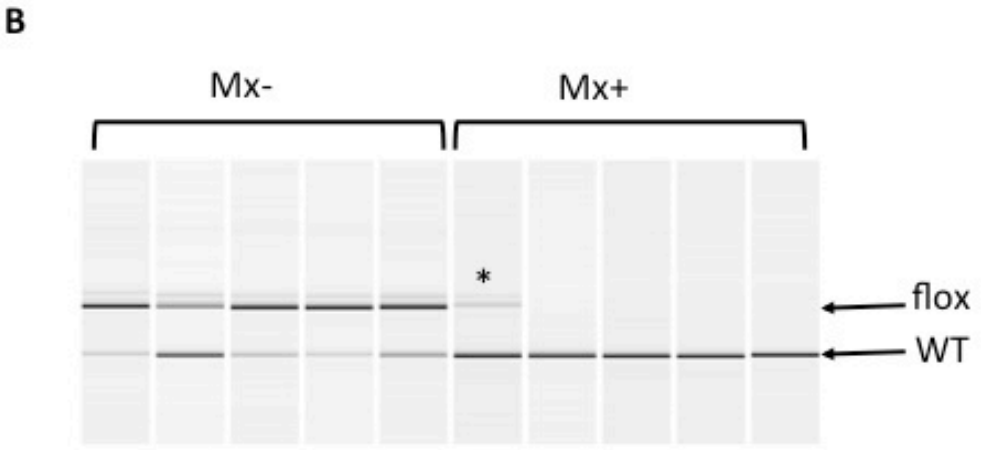
Supp Fig 1


JAK2+/+
Percent in PB
80
60
40
20
0
Pre
14
35
55
Days post-plpC
CD45.1
CD45.2
JAK2-/-
Percent in PB
100
80
60
40
20
0
Pre
14
35
55
Days post-plpC
CD45.1
CD45.2

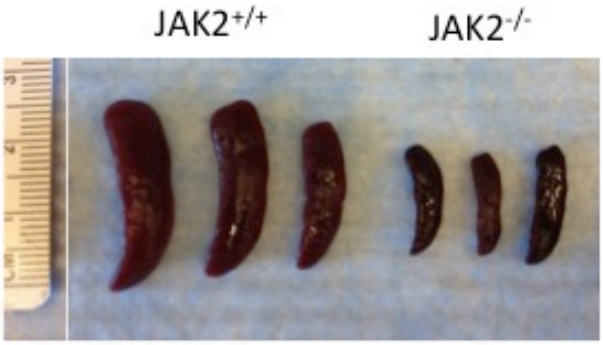
Supp figure 2



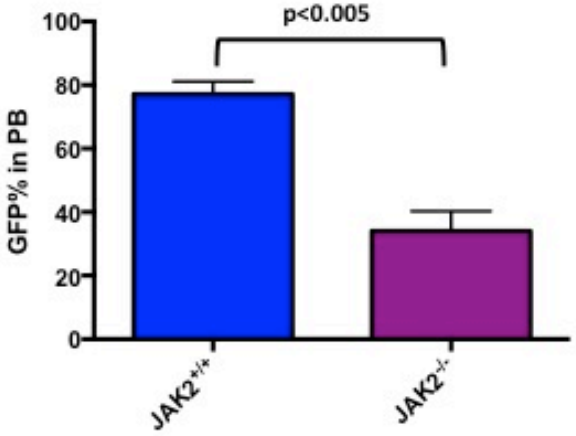


Supp Fig 3

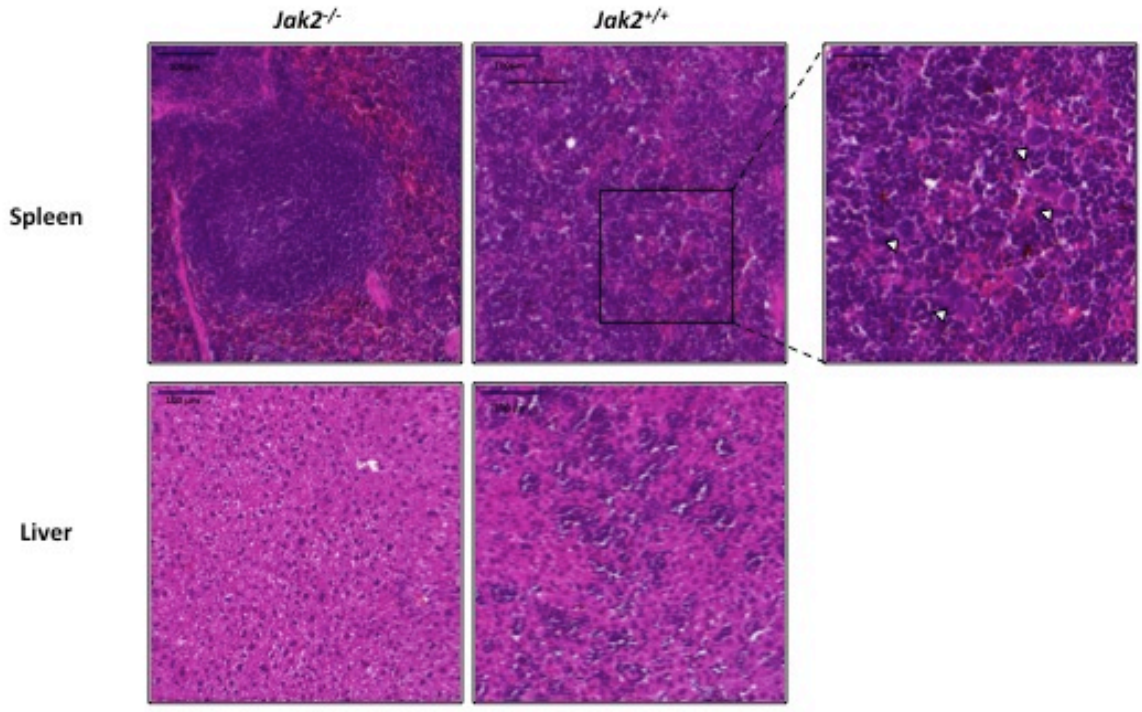
A



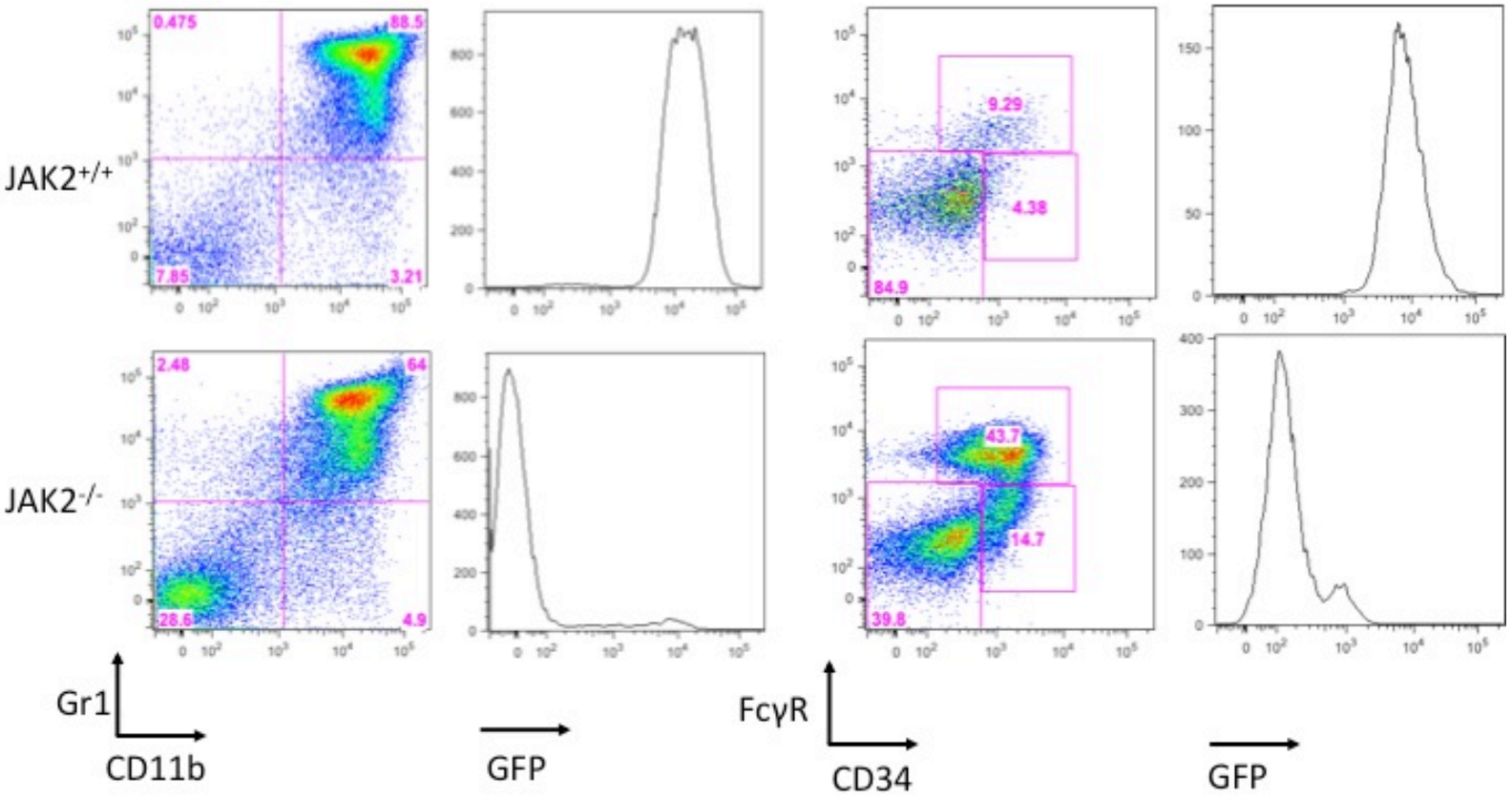
B



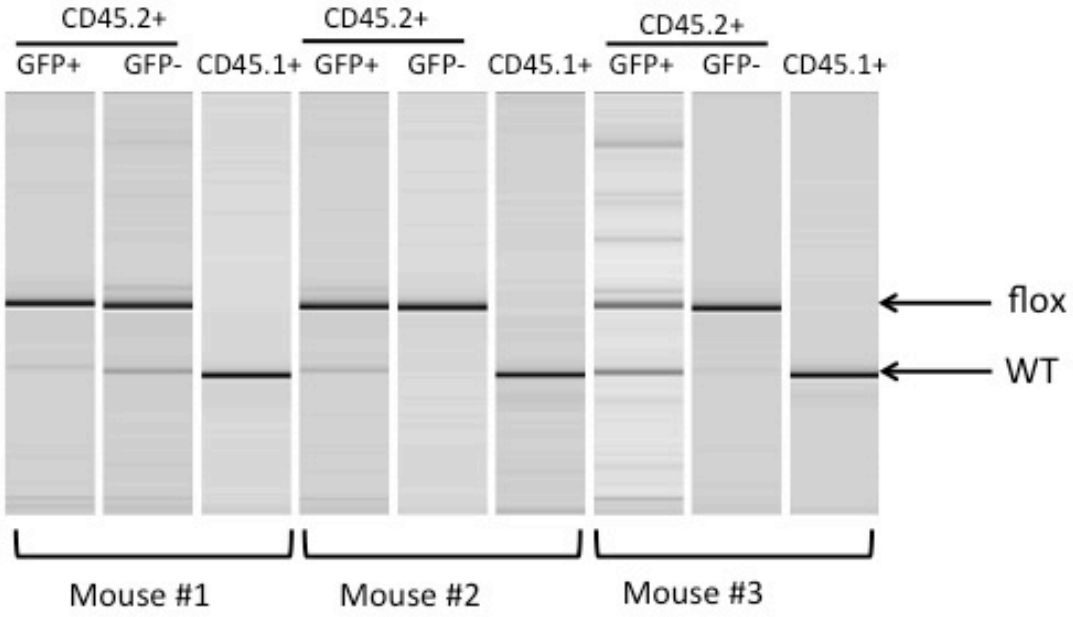
Supp Fig 4



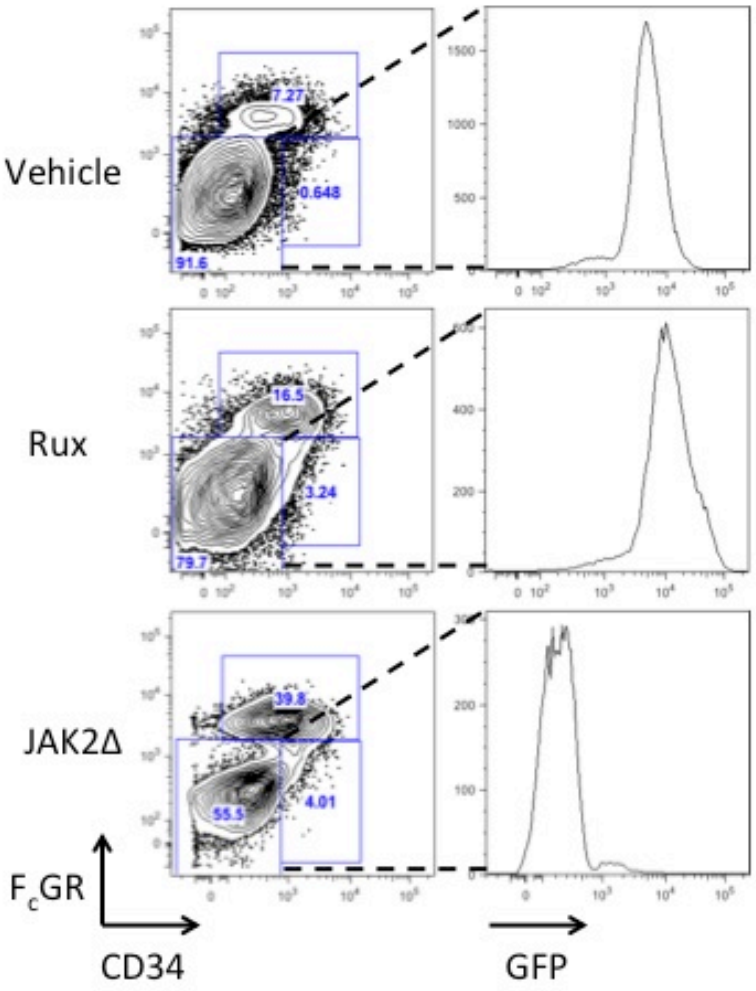
Supp Fig 5


JAK2+/+
JAK2-/-
0.475
88.5
7.85
3.21
2.48
64
28.6
4.9
9.29
4.38
84.9
43.7
14.7
39.8
Gr1
CD11b
GFP
FcγR
CD34
GFP

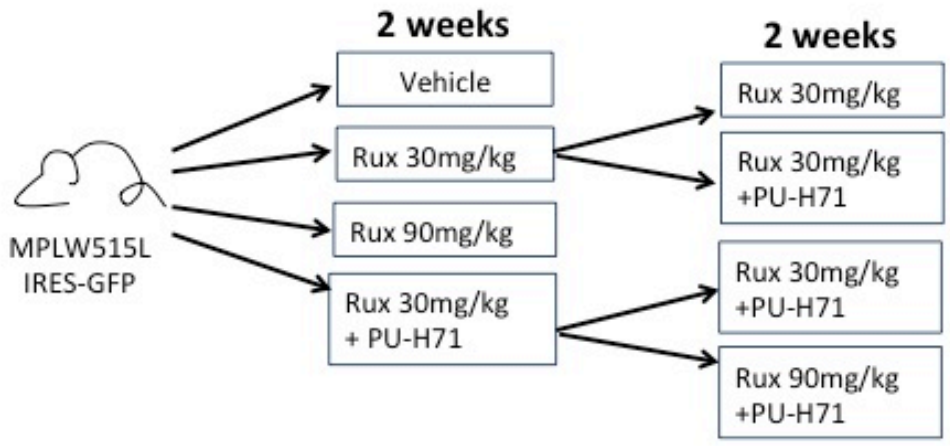
Supp fig 6


CD45.2+
CD45.2+
CD45.2+
GFP+
GFP-
CD45.1+
GFP+
GFP-
CD45.1+
GFP+
GFP-
CD45.1+
flox
WT
Mouse #1
Mouse #2
Mouse #3

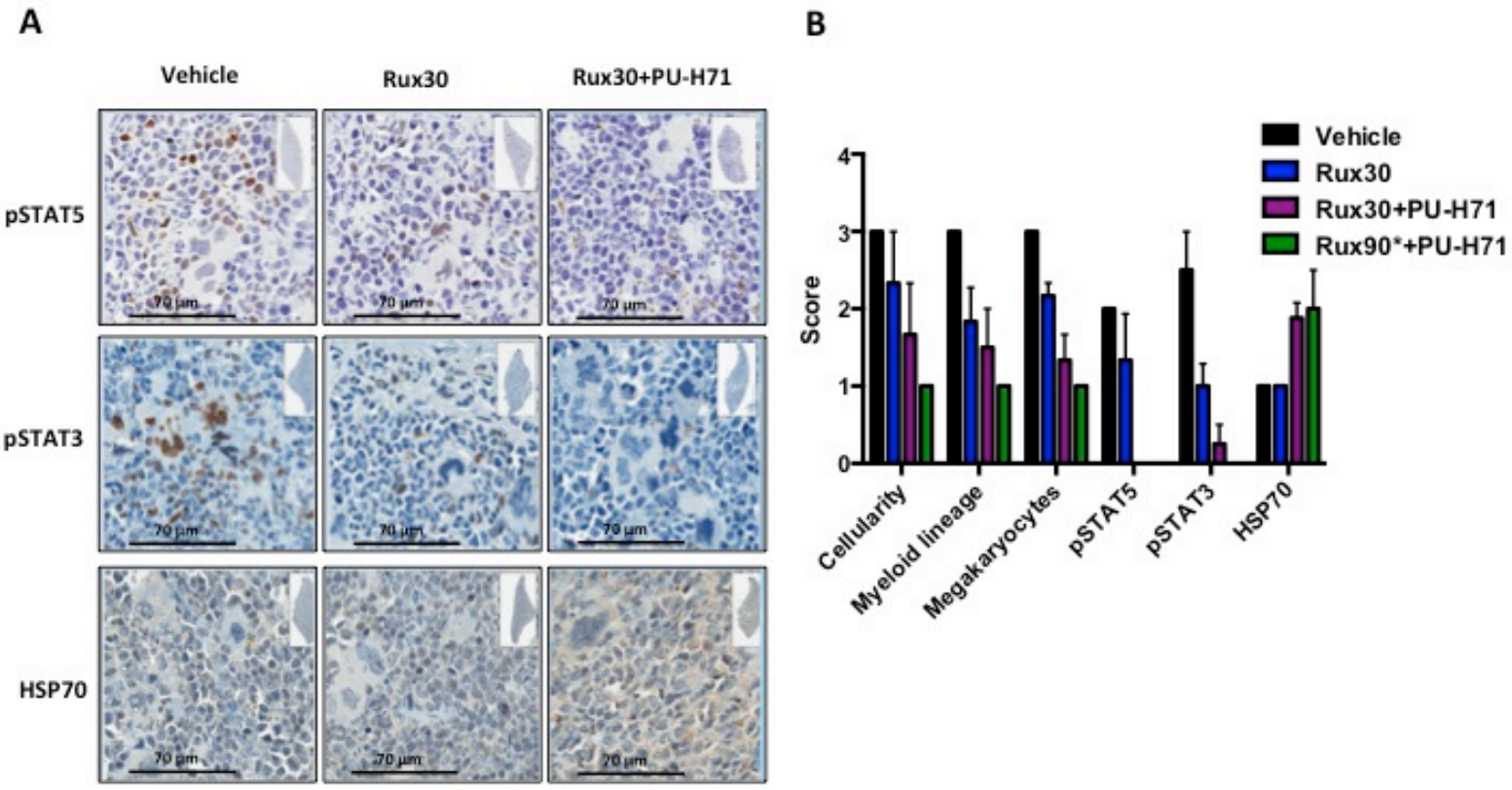
Supp Fig 7


Vehicle
7.27
0.648
91.6
Rux
16.5
3.24
79.7
JAK2Δ
39.8
4.01
55.5
F$_c$GR
CD34
GFP

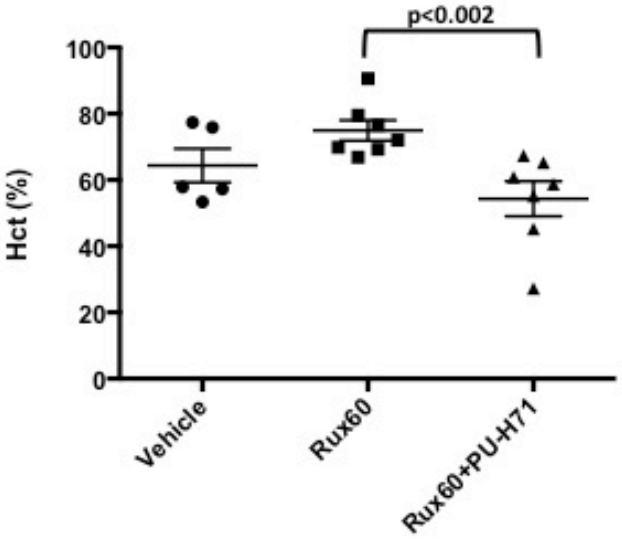
Supp Fig 8

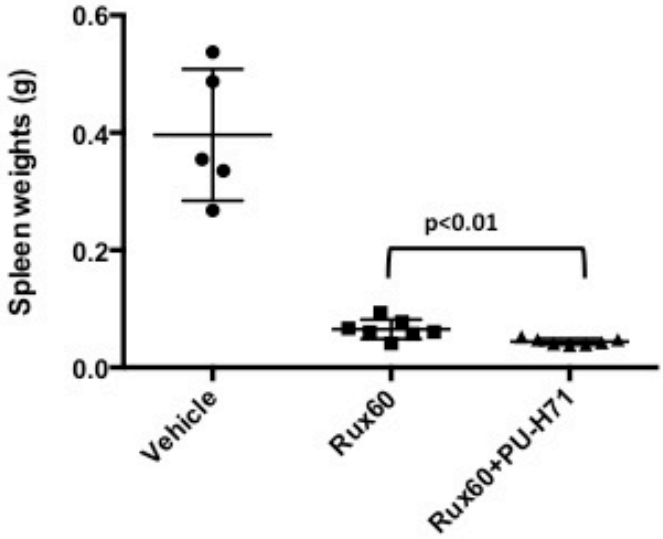

2 weeks
2 weeks
Vehicle
Rux 30mg/kg
Rux 90mg/kg
Rux 30mg/kg
+ PU-H71
MPLW515L
IRES-GFP
Rux 30mg/kg
Rux 30mg/kg
+PU-H71
Rux 30mg/kg
+PU-H71
Rux 90mg/kg
+PU-H71

Supp Fig 9

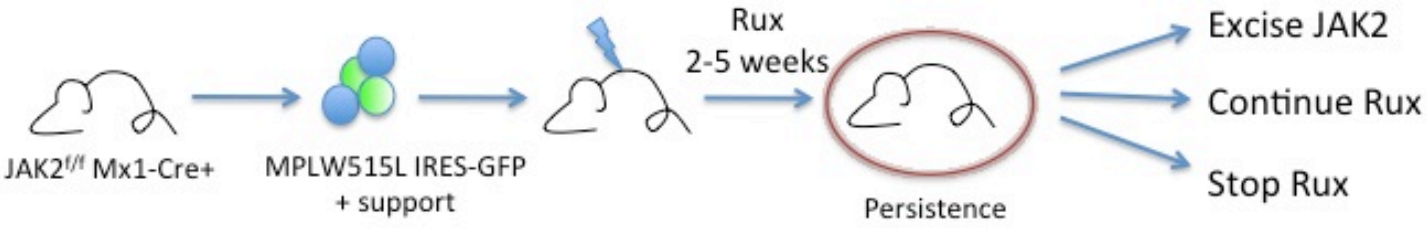

A
Vehicle
Rux30
Rux30+PU-H71
pSTAT5
pSTAT3
HSP70
70 µm
B
Vehicle
Rux30
Rux30+PU-H71
Rux90*+PU-H71
Score
0
1
2
3
4
Cellularity
Myeloid lineage
Megakaryocytes
pSTAT5
pSTAT3
HSP70

Supp Fig 10

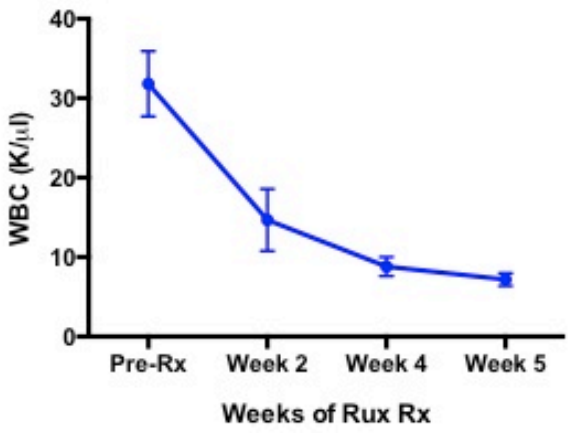

p<0.002
Hct (%)
100
80
60
40
20
0
Vehicle
Rux60
Rux60+PU-H71

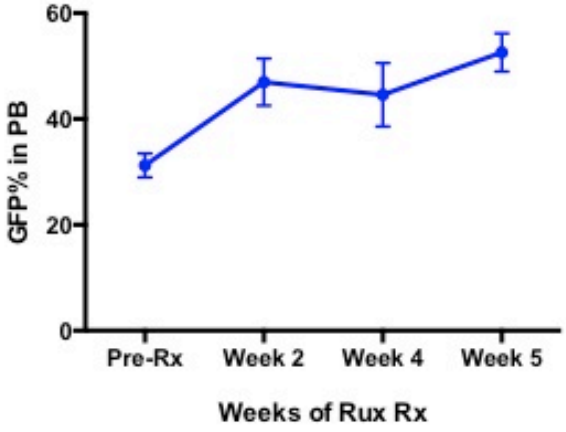

Spleen weights (g)
0.6
0.4
0.2
0.0
p<0.01
Vehicle
Rux60
Rux60+PU-H71

Supp. Fig. 11


JAK2f/f Mx1-Cre+
MPLW515L IRES-GFP
+ support
Rux
2-5 weeks
Persistence
Excise JAK2
Continue Rux
Stop Rux

Supp. Fig. 12


40
30
20
10
0
WBC (K/μl)
Pre-Rx
Week 2
Week 4
Week 5
Weeks of Rux Rx


60
40
20
0
GFP% in PB
Pre-Rx
Week 2
Week 4
Week 5
Weeks of Rux Rx

Supplementary Figure legends

Supp. Fig. 1: Loss of JAK2 results in decrease in peripheral blood chimerism in *MPLW515L*-transduced mice. CD45.2 *Jak2*$^{f/f}$ *Mx1-Cre*+ and *Mx1-Cre*- bone marrow was transduced with retroviral *MPLW515L-IRES-GFP* and transplanted into lethally irradiated recipients along with equal number of CD45.1 support bone marrow. Deletion of *Jak2* following engraftment leads to significant reduction in peripheral blood chimerism.

Supp. Fig. 2: Residual disease is due to cells with intact *Jak2*. One mouse from the Mx1-Cre+ cohort had (A) an enlarged spleen and higher proportion of GFP+ cells in peripheral blood as compared to other mice in this cohort (marked by an *). (B) DNA from peripheral blood from this mouse revealed incomplete excision (marked by *)

Supp. Fig. 3: JAK2 is required for MPN disease maintenance. Deletion of *Jak2* following disease establishment resulted in significant reduction in (A) spleen size and (B) mutant allele burden in peripheral blood (PB) in terms of GFP positive cells.

Supp.Fig. 4: Deletion of *Jak2* leads to reduced pathologic myeloproliferation. Higher magnification images (20X and 40X) showing restoration of splenic architecture and reduced myeloid infiltration in the liver. Abnormal megakaryocytes in the spleen are indicated by white arrowheads.

Supp. Fig. 5: Loss of JAK2 leads to reduction in progenitors and mature myeloid cells in bone marrow. Deletion of *Jak2* in *MPLW515L*-transduced mice led to a decrease in CD11b+Gr1+ cells and megakaryocyte-erythroid progenitor (MEP) populations along with a dramatic reduction in contribution of GFP positive cells to these populations. Representative flow cytometry graph from data is presented.

Supp. Fig. 6: Residual GFP+ cells have unexcised *Jak2*. DNA was isolated from sorted GFP+ and GFP- cells as well as CD45.1 cells from bone marrow from three Mx1-Cre+ mice. GFP+ cells retained JAK2 floxed allele, which was not detectable in unsorted cells (data not shown) indicating that residual GFP+ disease cells had incomplete excision of JAK2.

Supp. Fig. 7: Loss of JAK2 leads to significant reduction in MEP population compared to ruxolitinib treatment. Deletion of *Jak2* in *MPLW515L*-transduced mice led to a significant decrease in GFP positive progenitor population, which is not observed with JAK inhibitor treatment. Representative flow cytometry graph from data is presented.

Supp. Fig. 8: Schematic of JAK and HSP90 inhibitor trial design. *MPLW515L*-transduced mice were randomized to five groups as shown. After 2 weeks of treatment, 75mg/kg PU-H71 was administered in combination with ruxolitinib to a subset of ruxolitinib 30mg/kg arm. At the same time point, the dose of a subset of the ruxolitinib 30mg/kg + PU-H71 combo arm was increased to ruxolitinib 90mg/kg.

Supp. Fig. 9: JAK/HSP90 inhibitor combination therapy results in potent STAT inhibition and histopathological improvements (a) Immunohistochemical analysis of spleen from vehicle and inhibitor treated mice shows reduction of pSTAT3 and pSTAT5

signaling and induction of HSP70 in combination treated arm. (b) Quantification of spleen histopathological analysis showing improvements in myeloproliferation with combination treatment in ET/MF model.

Supp. Fig. 10: JAK/HSP90 inhibitor combination treatment is efficacious in *Jak2V617F* knockin model of MPN. Bone marrow from a *Jak2V61F* knockin donor mouse was transplanted into lethally irradiated recipients, which were treated with vehicle, 60mg/kg ruxolitinib or 60mg/kg ruxolitinib + 75mg/kg PU-H71 for 28 days. Mice receiving combination treatment had significantly lower hematocrits and spleen weights as compared to mice receiving ruxolitinib monotherapy.

Supp. Fig. 11: Schematic showing experimental design of Jak2 deletion in inhibitor persistence model. Bone marrow from *Jak2*$^{f/f}$ *Mx1-Cre+* mice was transduced with *MPLW515L* retrovirus and transplanted into lethally irradiated recipients. Following disease establishment, ruxolitnib treatment was initiated in all mice. After two weeks of drug treatment, *Jak2* was deleted in a subset of mice while all other mice continued to receive ruxolitnib. After five weeks of ruxolitinib therapy, treatment was stopped in all mice and they were monitored for an additional three weeks.

Supp. Fig. 12: Long-term ruxolitinib treatment does not reduce allele burden. Five weeks of ruxolitinib treatment reduces blood counts but does not lead to a reduction in GFP positive cells in peripheral blood.